lack of sufficient quantities of protein during those early stages and the time required to perform the ITC experiments on large numbers of potential ligands (or protein constructs) of interest. Traditionally 50 -1500 μg of protein has been consumed to complete an ITC experiment and completing each experiment could require two hours or more. Data presented we will describe the characteristics of a new miniaturized, ultrasensitive ITC that has been designed to push back these limitations allowing ITC to be effectively utilized at earlier stages of the drug discovery and development process.

3264-Pos Structure-Activity Relationship Between Lipopolysaccharide And An Antimicrobial Fragment Of Human Cathelicidine

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Board B567

Human cathelicidine hCAP18 is a potential lead structure for new antibiotics to overcome the rising number of pathogenic multidrug resistant bacterial strains.

In the case of Gram-negative bacteria, the first contact sites of antimicrobial peptides are the molecules of the outer leaflet of the outer membrane, mainly Lipopolysaccharide (LPS). LPS is able to induce an inflammatory response, which may cause the fatal septic shock.

To investigate the structure-activity relationship between LL-32, an active fragment of hCAP18, and LPS, especially the influence of the core oligosaccharides, we used fragments of LL-32, lacking some amino acids residues, and *Salmonella enterica* serovar Minnesota strains differing in the length and charge of the core oligosaccharides. *In vivo* hCAP18 is processed to even shorter peptides than LL-32, so these fragments might play an important role in the immune system.

We determined the antibacterial and antiinflammatory activities of these peptides against the *S. enterica* strains. Furthermore, we measure the interaction of the fragments with membrane-models composed of different LPS with physical methods. Binding of LL-32 to LPS and bacteria was investigated by ITC and by measuring the Zeta-potential. After binding to LPS, LL-32 induce lesions or disrupt the membrane by micellisation. We measured the ability of the fragments to fuse LPS-aggregates using a spectroscopic assay based on Förster Resonance Energy Transfer. Furthermore, we determined the size of LPS-aggregates in dependence of the LL-32 concentration. FACS measurements using fluorescently-labelled LL-32 on bacteria showed that LL-32 bind instantly to bacteria leading to a permeabilisation of the membranes.

Our data allows to explain the different biological activities of LL-32 by means of biophysical data. Furthermore, the different behaviour of the used fragments allows identifying specific residues in the AA sequence of LL-32, which are important for its activities.

3265-Pos Dual Mechanism Of Bacterial Lethality For A Cationic Sequencerandom Copolymer Mimicking Host-defense Antimicrobial Peptides

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Board B568

Flexible sequence-random polymers containing cationic and lipophilic subunits, which can be induced by a bacterial membrane surface to adopt globally amphiphilic irregular structures, have been synthesized (Mowery, B.P., *et al.*, submitted for publication). These polymers are mimics of antimicrobial host-defence peptides, which act by disrupting bacterial membranes. We have studied one such copolymer, BPM2-39B, having an average length of 21 residues, which is very active against both Gram positive and Gram negative bacteria, with regard to its ability to disrupt model and bacterial membranes. Our findings show that at very low concentrations, comparable to their MIC values, it is able to permeabilize, in a highly cooperative fashion, model membranes mimicking the lipid composition of *E. coli, S. aureus* and *B. subtilis*. It is ineffective against zwitterionic membranes, which explains its low hemolytic capacity. Both DSC and ITC indicate that it is capable of binding as well as segregating anionic lipids, forming anionic lipid-rich and anionic lipid-poor domains. Experiments with the *E. coli* mutant ML-35p, indicated that permeabilization in Gram negative bacteria is biphasic; at low concentrations (up to 25 μg/mL) the polymer is capable of permeabilizing the outer membrane, which is blocked at higher concentrations of polymer. Experiments with *E. coli* K-12, showed that at very low concentrations the polymer is also able to reach and disrupt the inner cytoplasmic membrane. Despite the fact that at higher concentrations the polymer does not permeate the inner membrane, it associates with the negatively charged LPS layer (or LTA in gram positive bacteria), with lethal consequences for the organism. We propose then a dual mechanism of bacterial killing by flexible sequence-random copolymers, which differ at low and high concentrations of polymer.

Regulatory Networks & Systems Biology - I

3266-Pos Closing The Loop: Towards A Comprehensive View Of Action At A Distance In Transcriptional Regulation

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Board B569

DNA architecture plays a key role in determining spatial and temporal patterns of gene expression. This architecture encompasses both the nucleotide sequence (i.e., the information content)

and the physical state of the DNA such as its spatial organization and mechanical properties. We explore transcriptional regulation by DNA looping in the *lac* operon, where transcriptional control is realized by the simultaneous binding of Lac repressor to two binding sites separated by hundreds of base pairs on the DNA. We develop a statistical mechanical model to quantify the *in vivo* energy cost of different DNA conformations in bacteria, which allows us to extract mechanical properties of DNA and to compare completely different regulatory systems such as the *lac* operon and the arabinose operon as well as to explain the results of single molecule looping experiments. By controlling DNA architectural properties such as the length of the intervening DNA and its sequence-dependent flexibility we generate a set of falsifiable predictions. We present our recent experimental efforts aimed at systematically probing these predictions both *in vivo* and *in vitro. In vitro*, we use the tethered particle method to systematically explore the role of DNA length and flexibility in dictating looping probabilities. *In vivo,* we use the fold-change in gene expression for the same set of sequences used in our *in vitro* experiments to compare and contrast the *in vitro* and *in vivo* pictures of regulation.

3267-Pos Ab Initio Thermodynamic Modeling Of Distal Multisite Transcription Regulation

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Board B570

Transcription regulation typically involves the binding of proteins over long distances on multiple DNA sites that are brought close to each other by the formation of DNA loops. The inherent complexity of assembling regulatory complexes on looped DNA challenges the understanding of even the simplest genetic systems, including the prototypical lac operon. Here we implement a scalable approach based on thermodynamic molecular properties to model ab initio systems regulated through multiple DNA sites with looping. We show that this approach applied to the lac operon accurately predicts the system behavior for a wide range of cellular conditions, which include the transcription rate over five orders of magnitude as a function of the repressor concentration for wild type and all seven combinations of deletions of three operators, as well as the observed induction curves for cells with and without active CAP. Our results provide new insights into the detailed functioning of the lac operon and reveal an efficient avenue to incorporate the required underlying molecular complexity into fully predictive models of gene regulation.

3268-Pos Monte Carlo simulation elucidates the mechanism of antigen affinity discrimination by B cells

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Board B571

We have developed a Monte Carlo-based stochastic simulation model to investigate the mechanisms of differential B cell signaling upon ligation with antigens of varying affinity. Recent experiments have revealed that B cell signaling is modulated through the immune synapse upon ligation with membrane bound antigens. The immune synapse is an ordered structure consisting of segregated BCR/Antigen and LFA-1/ICAM-1 molecules that is formed at the cell-cell contact area. For a given antigenic affinity, our numerical experiments gave rise to stochastically varying levels of activated signaling molecules such as phosphorylated BCR Igα/Igβ and Syk molecules. We determine the probability distribution of the major signaling molecules from many runs of our stochastic simulation as we vary the antigen affinity in increments of an order of magnitude. Such probability distribution of downstream signaling molecules of B cell signaling determines how affinity discrimination is achieved by signaling through immunological synapses. For soluble antigens, a diffusion-dependent coarse-grained model of receptor clustering (capping) at the B cell surface yields similar stochastically varying levels of signaling molecules and results in affinity discrimination through receptor cap formation.

3269-Pos Modeling complex networks: Integrating rules (BioNetGen) and data mining (BioPAX ontology) into the *Virtual Cell* framework

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Board B572

A combinatorial complexity often arises when detailed quantitative models of intracellular networks are being sought. Proteins are composed of functional modules (e.g. binding sites, phosphorylation sites) and can thus exist hundreds of different forms. Many or all of them must be accounted for in a quantitative model to simulate the time course for receptor-mediated signaling. Simple models of biochemical kinetics accounting for dozens of different molecular species are a norm; models accounting for hundreds of species and reactions are no longer rare. When details of all functional forms are being included, this number can easily increase by a few orders of magnitude, and validation, visualization, and understanding can become virtually intractable. A solution for this challenge is provided by 1) automatic extraction from pathway databases re-usable model components for quantitative models, and 2) rules of interaction based on protein modularity. This way, quantitative models of large, complex networks can be assembled from separately constructed, validated, and visualized components, either directly or via rules. To implement this strategy, we have combined the strength of several related technologies: the Biological Pathways Exchange (BioPAX) ontology, the Systems Biology Markup Language (SBML) format, the BioNetGen rule-based description of molecular interactions, and the Virtual Cell (VCell) modeling and simulation software framework. Two approaches are used to generate models without manual specification of each and every species and reactions. First is using BioPAX data imported from BioPAX-compati-

ble databases, providing for a well-documented biological identification for each element of the model. Second is to specify a model in the form of bio-molecular interaction rules that generate a biochemical reaction network. This approach has been implemented in general-purpose software, BioNetGen, and recently has been implemented as a BioNetGen@VCell application.

3270-Pos Modeling Prostate Cancer as Intrinsic Functional Disease

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Board B573

A mathematical model for cell growth, apoptosis and their molecular regulations for prostate cells and infiltrated immune cells, generally considered relevant to prostate cancer, is constructed based on molecular biology experimental observations. Stochastic dynamical analysis with adaptive landscape potential functions is used to study the dynamics of such a molecular and cellular endogenous interaction network. This network is found to possess multiple robust functional states with evident biological implications. Among these states are ones with higher metabolic activities, high concentration of self-generated growth factors along with different growth and apoptotic behaviors. Under possible physiologic conditions, such functional modes may be needed in response to change of conditions such as stress and injury. Prostate cancer is thus proposed as an 'intrinsic disease", a failure to choose proper operating modes by its endogenous dynamical network. The multiple functional states and their dynamics may be represented by a functional landscape quantified by a potential function constructed using a recently developed mathematical method. In this representation, to cure an intrinsic disease in an efficient way is equivalent to switching from an incorrect functional state to a desired one by navigating along the least difficult routes in the functional landscape, those passing through saddle points or passes. The mathematical results are also consistent with the observed spontaneous neoplastic transformation and heterogeneity of the prostate cancer. The main features of the present mathematical model appears shared by other cancers.

3271-Pos Marine vesicles: Adhesion based detection of vesicles-like microparticles in seawater

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Board B574

Abiotic aggregation of biopolymers in seawater has the potential to greatly affect carbon conversion in ocean systems, self-assembly of dissolved biocompounds into vesicles being a significant root by which organic matter may enter the aquatic food chain. Vesicles-like microparticles (VLM) have been for the first time identified in seawater by amperometric detection of single adhesion events of vesicles at the electrode/seawater interface.

In the northern Adriatic Sea concentrations of VLM (sizes$\geq$3 μm) in seawater varied from 10^5 to 2$\times$10^7 L. We recorded the pronounced spatial and temporal variability of VLM, depending on the season, depth and trophic gradient. AFM images of submicron particle fractions revealed round patches, 1200 to 50 nm in diameter, of surprisingly uniform height, 1.2 - 1.4 nm, which are typical values reported for polysaccharide single or double molecular chain heights. VLM production recorded during the diatom bloom experiment shows an extraordinary capacity of extracellular polysaccharides for self-organization, reaching in seawater a level of self-assembly resembling that of organelles (that is, liposomes) in living cells.

Because such ordered structures are indicative of directed processes, it cannot be ruled out that MLV catalyse very specific biochemical processes associated with compartmentation, selective transport, and information structuring that are known for biological systems.

3272-Pos Multisite Phosphorylation Governs Oscillation Of A Three-Protein Circadian Clock

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Board B575

The simple circadian oscillator found in cyanobacteria can be reconstituted in vitro using three proteins-KaiA, KaiB and KaiC. The total phosphorylation level of KaiC oscillates with a circadian period, but the mechanism underlying its sustained oscillation remains unclear. Here we show that four forms of KaiC differing in their phosphorylation state appear in an ordered pattern arising from the intrinsic autokinase and autophosphatase rates of KaiC and their modulation by KaiA. Kinetic and biochemical data indicate that one of these phosphoforms inhibits the activity of KaiA via interaction with KaiB, providing the crucial feedback that sustains oscillation. A mathematical model, tightly constrained by experimental data, quantitatively reproduces the circadian period and the distinctive dynamics of the four phosphoforms.

3273-Pos Signaling Dynamics Of 'Inside-out' Integrin Activation During Lymphocyte Trafficking

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Board B576

Blood borne lymphocytes homing to peripheral sites of immune challenge require rapid activation of adhesion receptors to ensure localized cell arrest and emigration into surrounding tissues. To precipitate the transition from rolling to firm adhesion, endothelial-displayed chemokines bind specific G-protein coupled receptors on the lymphocyte surface, initiating a signal cascade which in turn up-regulates the adhesiveness of resting integrins. Using a computational approach to model biochemical transduction events, we address how the intracellular organization of such 'inside-out' signaling networks impacts the efficiency of lymphocyte arrest. In particular, we focus on the regulation of the β_2 integrin, LFA-1, whose affinity is modulated by the small GTPase Rap1. Our stochastic framework explicitly simulates the temporal and spatial evolution of all molecular species downstream of the chemokine receptor leading to Rap1 and subsequent LFA-1 activation. Employing this dynamical model, we predict how parameters governing chemokine recognition (i.e. affinity, off-rate, density) affect the propagation of the inside-out signal. The predicted dynamics are calibrated against experimental measurements of second messenger release and affinity up-regulation reported from a novel micropipette stimulation-adhesion assay. Together, these studies illustrate how local membrane-proximal signaling might account for the extraordinary speed ($<$ 1s) with which chemokines modulate lymphocyte adhesion, and highlight how cells distinguish between chemokines with variable agonist potency.

3274-Pos Investigation of Bacteria Communication at the Single-Cell Level in a Microbioreactor

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Board B577

Bacteria communicate with one another using extra-cellular signaling molecules AIs (auto inducers). The information supplied by AIs is critical for synchronizing the activities of the cell population. We have quantitatively studied how *Vibrio harveyi* detect and respond to AIs, by measurements at the single-cell level in a microliter-volume microbioreactor, made of poly (dimethysiloxane) (PDMS) and glass. To accurately measure the contribution of AIs, we constructed strains with a fluorescence reporter for AIs. Such strains also have another fluorescence reporter under constitutive promoter, which serves as internal standard. With these strains we observed how individual bacteria's respond to step changing in concentration of AIs. We analyzed the strength and time-delay of the bacteria's response as a function of AI concentration.

3275-Pos Investigating Bacterial Quorum Sensing at Single Cell Level

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Board B578

Quorum sensing (QS) is an important process for cells to communicate and to coordinate their activities in response to changes in their population densities. To explore the spatial-temporal development of quorum sensing, we have investigated the behavior of the bioluminescent marine bacterium *Vibrio Harveyi* at single cell level. The cell produces, secretes and detects extracellular signaling molecules called autoinducers (AIs). The AI receptors are distinct membrane-bound two-component signaling proteins. Under low cell density (LCD) conditions, these receptors act as kinases in the absence of AIs, and phosphate is transferred to the response regulator protein, LuxO. LuxO-P activates the transcription of multiple small RNAs (sRNAs) called Qrr1-5, which bind to and destabilize the mRNA of the central QS regulator LuxR. Under high cell density (HCD) conditions, the interaction of receptors with AIs switches the receptors to phosphatase mode, leading to dephosphorylation of LuxO. LuxO can no longer activate the Qrrs and LuxR is not repressed. Previous studies suggest that LuxR can positively regulate the Qrrs in a LuxO-dependent manner. Here, we examined this feedback regulation using a straing that contains a *qrr*4-promoter *gfp* fusion and bioluminescence as readout for LuxR activity. Using light microscopy at the single-cell level, we observed cells responding to density variations from HCD to LCD as well as from LCD to HCD. By digitizing images of the growing microcolonies, we traced out the individual lineages of each cell in a microcolony. The fluorescence and luminescence signals were processed to optimize the resolution of the components in the pathway and to compare between lineage lines. We observed that upon changing from HCD to LCD, cells with more LuxR seemed to have lower Qrr4 promoter activity, which was in contrast to the previous studies that LuxR positively regulates Qrrs.

3276-Pos Development of Numerical Matrix Method for Biochemical System Identification

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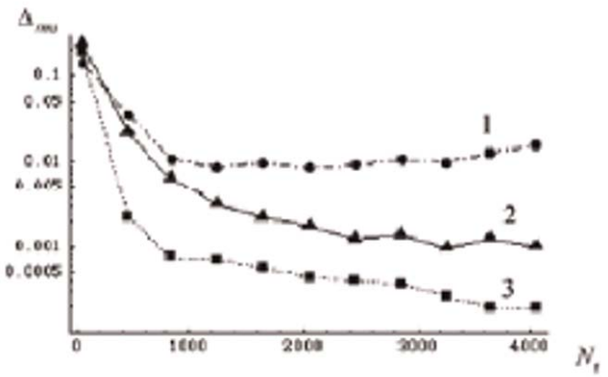
Board B579

The Numerical Matrix Method (NMM) is a flexible set of methods for determination of rate constants and reaction mechanism from complex kinetic data (Karnaukhov, A., et al., Biophys. J., 2007, *92*, p. 3459–3473). Rate constant reconstructions can be made from time series data in the absence of a specific kinetic model. The accuracy of rate constant determination in NMM significantly depends on the quantity of prior information about the system, the number of time points and noise level of experimental data. In addition, the accuracy depends on the method of numerical differentiation of the time series data. Numerical differentiation based on the polynomial approximation (curve 3 in Figure 1 below) provides the lowest Δ_{rms} from original rate constants compared to two traditional finite-difference methods (curves 1 and 2). Additional

applications of the method of numerical differentiation based on the polynomial approximation include the possibility of the robust error analyses of rate constants value determined by application of NMM. This error analyses will be used for error analysis of rate constants determined using NMM from experimental Fluorescence Correlation Spectroscopy data.



3277-Pos Radical Pair-Based Magnetoreception

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Board B580

Every season many species of birds migrate due to an endogenous program that is designed to propagate the species through breeding and provision of larger food sources. The mystery of this biological phenomenon is how the birds are able to navigate to their new environment. One source of directional information that is always present is the Earth's dipolar magnetic field, which magnetic field experiments have shown that birds can use to determine migratory directions. However, the lack of an obvious site for a magnetic sensor, combined with the ability of magnetic fields to pass through all tissue has prevented the discovery of the mechanism responsible for the detection of Earth-strength fields. Two primary models have risen to the forefront of magnetoreception research: magnetite and radical pairs. In the radical pair model, the magnetic field affects a photochemical reaction step that involves light-induced creation of an intermediate pair of radicals. Recent experiments suggest that the blue-light receptor cryptochrome, which has been discovered in birds, plants, and other animals, is a promising candidate for a photomagnetoreceptor. Its presence in plants allows one to apply molecular biological and genetic approaches in order to determine the molecular basis of photochemical magnetoreception. Here, we report experimental measurements of magnetic field effects on hypocotyl growth in *Arabidopsis thaliana.* We determine the detection threshold of static magnetic field effects via dose-response curve measurements. We also investigate the effects of combined oscillating and static magnetic fields to obtain information about the chemical nature of the magnetosensitive reaction step through resonance effects. The results, combined with collaborative studies at the protein level, are compared to a conceptual model of the photochemical magnetic detection mechanism in cryptochromes with the goal of explaining how small magnetic effects in one reaction step can lead to stable physiological responses.

3278-Pos Systems Biology Of Compartmentalized cAMP Signaling In Cardiac Myocytes

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Board B581

The diffusible second messenger cAMP plays a critical role in regulating cardiac myocyte function. While it is often assumed that receptor activation produces a uniform change in cAMP levels throughout the entire cell, this does not easily explain many experimental observations. In the present study, a quantitative computational approach was used to further test the hypothesis that cAMP signaling in cardiac myocytes is compartmentalized. The model used incorporates existing kinetic data on the signaling pathways involved in regulating cAMP production and degradation into a theoretical cell consisting of three different compartments:

1. a subsarcolemmal space associated with caveolar membrane domains of the cell that are enriched in type II protein kinase A (PKA-II);
2. a subsarcolemmal space associated with cholesterol-rich lipid rafts that do not include caveolin; and
3. a bulk cytoplasmic compartment that makes up >90% of the cytosolic volume.

The behavior of the model was previously validated using a PKA-II FRET-based biosensor to estimate cAMP levels in the caveolar domain of adult ventricular myocytes (Biophys J *92*:3317–31, 2007). In the present study, we compared the behavior of the model with cAMP responses detected by a freely diffusible Epac2 FRET-based biosensor. By assuming that a small but significant fraction of receptor-dependent signaling occurs in the plasma membrane associated with the bulk cytoplasmic compartment, the new version of the model suggests that responses detected by the Epac2-based probe correlate closely with cAMP levels in that domain. These results demonstrate that the model is able to accurately describe the significant differences in cAMP concentration that exist between the caveolar and bulk cytoplasmic domains both under basal conditions as well as in response to receptor activation.

Bioinformatics

3279-Pos Prevalence of EH1 Motifs in Hox and Fox Domain Containing Transcription Factors from the Sponge *Suberites domuncula*

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